Study 5

Skin Sensitization Test in Guinea-Pigs, April 21, 1998

[redacted]

"SKIN SENSITIZATION TEST IN GUINEA-PIGS"

Maximization test

[redacted] EXP. No. [redacted]

EEC Guideline (B.6)
OECD Guideline (406)

Issued on April 21, 1998

SPONSOR

[redacted]

PERFORMING LABORATORY

[redacted]

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[redacted]

EXP. No. [redacted]

TITLE OF THE STUDY

"Skin sensitization test in guinea-pigs treated with the test article [redacted].

PURPOSE OF THE STUDY

Assessment of the contact sensitizing potential of the test article.

TEST METHOD

The Magnusson's maximization test was followed (1, 2, 3).

The test method is in accordance with method B.6, Annex V to Directive 67/548 (EEC Directive 96/54, EEC Official Jurnal, No. L 248, September 30, 1996) and with Organization for Economic Cooperation and Development (OECD) Guidelines (section 4, subpart 406, Paris 1981 and subsequent updatings).

PRINCIPLE

Following initial exposure to the test article (the "induction" period), the animals are subjected, approximately two weeks after the last induction exposure, to a "challenge" application of the test compound in order to establish whether a hypersensitive state has been induced.

Sensitization is determined by examining the skin reaction to the challenge exposure.

SENSITIVITY CHECK OF THE DUNKIN HARTLEY GUINEA-PIG

The sensitivity check of the Dunkin Hartley albino guinea-pig is normally verified at [redacted] twice a year, as indicated in the Guidelines, at 6-month intervals.

The data obtained in the last check performed are attached (see Attachment No. 1). These data show a clear sensitization of animals by 2,4-dinitrochlorobenzene (DNCB), therefore the strain of Guinea pigs used at [redacted] is suitable for detecting the possible sensitizing potential of test materials.

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[redacted] Exp. No. [redacted]

FOREWORD

On behalf of [redacted], authorized by the [redacted] Health Authorities (1-2) to conduct safety studies, has performed a skin sensitization test in guinea-pigs, [redacted] - Experiment No. [redacted], treated with the test article:

[redacted]

A sample of the substance used, along with relative documentation, is held in sufficient quantity in the [redacted] archives at the disposal of the Ministero della Sanità.

The undersigned declare that the experiment was conducted using the same batch of substance as that of the sample held on file.

For verification by the Ministero della Sanità, the undersigned moreover guarantee the identification and classification of all those materials, documents and recordings used in conducting the experiment, held on file for a period of at least 10 years from the date of this report. Following this time, they will be placed at the disposal of the Sponsor.

Dr. [redacted]

[redacted] Scientific and Operative Director

Ivrea, April 21, 1998

(1): **Pharmaceuticals:**
Authorization dated March 12, 1976 in accordance with "Circolare 73", May 16, 1974

(2): **Chemicals:**
Authorization in accordance with DPR 927/81 (D.M. dated January 7, 1988 published in G.U. No. 12, dated January 16, 1988).

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[redacted] Exp. No. [redacted]

QUALITY ASSURANCE STATEMENT

[redacted] Experiment number: [redacted]

Study title:

"Skin sensitization test in guinea-pigs treated with the test article [redacted]".

Studies of the type described in this report are conducted in a manner which involves frequent repetition of identical or similar procedures.

In compliance with the Principles of Good Laboratory Practice, at the time of this study, procedure-based inspections were made by the Q.A.U. of critical phases and procedures relevant to this type of study. For the inspection of any given procedure, studies were selected at random. All such inspections were reported promptly to the Study Director and to facility management.

This study was inspected on:

Dates of inspection/audit	Dates of report to Study Director and Management
February 16, 1998	February 16, 1998
April 17 and 18, 1998	April 18, 1998

This report has been audited by the Q.A.U. and was found to be an accurate description of such methods and procedures as were used during the conduct of the study and an accurate reflection of the raw data.

Date of final report audit: April 24, 1998

[redacted]

Head of Quality Assurance Unit

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[redacted] Exp. No. [redacted]

CERTIFICATION OF GLP COMLPIANCE

[redacted] Experiment No.: [redacted]

Study title: "**Skin sensitization test in guinea-pigs treated with the test article** [redacted]

I hereby confirm that this study was conducted in accordance with the OECD [C(81) 30 (final)], Principles of Good Laboratory Practice (GLP).

The Sponsor is responsible for GLP compliance of any information supplied.

These principles were adopted by the EEC and incorpored into EEC Directive 88/320, that was legally enforced by the [redacted] Health Authority [D.M. dated June 26, 1986 as published in G.U. No. 198, dated August 27, 1986 and D.L. January 27, 1992, No. 120 as published in G.U. (Supplement) No. 40, February 18, 1992].

The final report fully and accurately reflects the raw data generated during the conduct of the study.

This report consists of 34 pages.

[redacted] Study Director

Dr. [redacted]

Ivrea, April 24, 1998

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[redacted] Exp. No. [redacted]

SPONSOR IDENTIFICATION

[redacted]

SCIENTISTS INVOLVED IN THE STUDY

"Skin sensitization test in guinea-pigs treated with the test article [redacted]

[redacted] Study Director	Dr. [redacted]	
[redacted] Senior Scientist for General Toxicology	Dr. [redacted]	
Centralized Pharmacy Head	Dr. [redacted]	
Pharmacy Service Head	Dr. [redacted]	

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MATERIALS AND METHODS

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[redacted] Exp. No. [redacted]

PRODUCT IDENTIFICATION AND CHARACTERIZATION

Test article (supplied by the Sponsor)

Identification:	[redacted]
Batch No.:	[redacted]
Characteristics:	white wax/solid
Preparation date:	October 14, 1997
Expiry date:	December 2000
Storage conditions:	Room temperature
Purity:	>99%

The Sponsor reserves the right to divulge any other relevant data on test article characterization directly to Regulatory Agency(ies), when appropriate.

Vehicle characterization

Identification: deionized water

Adjuvant characterization

Identification:	Freund's complete adjuvant (FCA)
Batch No.:	86321 LA
Expiry date:	February 1999
Storage:	15-30°C, in the dark
Producer:	Difco Laboratories; Detroit Michigan-USA

PRELIMINARY TEST (Tolerability)

The preliminary test was performed on a total of 4 animals in order to select the highest concentration that causes mild irritation to be used in the induction phase and the highest concentration that proves not to be irritating for the challenge exposure.

Initially an aliquot of 0.1 ml of four different concentrations (2, 5, 10 and 15%) of the test article (vehicle = deionized water) were injected into four different areas of the shoulder region of two animals.

Three patches containing 0.3 ml of three different concentrations (15, 25 and 50%) of the test article (vehicle = deionized water) were applied for 24 hours in three different areas of the dorsal region of the same two animals treated intradermally.

The 50% concentration of the test article in deionized water was the highest concentration administrable to animals (see [redacted] internal communication dated February 5, 1998).

Twenty-four hours after the administration, the patches were removed and the animals were observed for up to 48 h for local reactions on the skin areas both of the intradermal injection and of the patch application.

All the four concentrations tested for intradermal injection resulted in eschar formation.

None of the three concentration assayed for the patch application were irritant.

On the basis of the results obtained, two additional animals were treated by intradermal injection with the test article concentrations of 0.1, 0.2 and 0.5% (for each concentration 0.1 ml was injected) in three different shoulder regions.

The 0.2 and 0.5% concentrations resulted in eschar formation, while the 0.1% concentration produced slight erythema.

Therefore in the experiment the test article was used at the concentration of 0.1% in the induction with the intradermal injection, at 50% in the booster (with sodium lauryl sulphate treatment in the previous day) and at 50% in the challenge application.

TEST ARTICLE FORMULATE PREPARATIONS

When necessary, an exact amount of [redacted] was weighed in a suitable container and made up to final volume with deionized water to obtain the concentration required.

For the injection in Freund's complete adjuvant (FCA) the test article was dissolved in water for injection at a concentration of 0.2% and then equal volume of FCA was added to obtain the concentration of 0.1% .

The test article formulates were prepared just prior to administration.

TEST SYSTEM

Species and strain:	Dunkin Hartley albino guinea-pigs
Justification for the selection of the test system:	Dunkin Hartley albino guinea-pig is the species generally recommended by Health Authorities as the experimental model for skin sensitization studies
Supplier:	[redacted] Shipping slip No. 00387, dated January 16, 1998 (preliminary study) Shipping slip No. 01195, dated February 13, 1998 (main study)
Number:	19 animals as follows: 10 animals for the treated group 5 animals for the control group 4 animals for the preliminary test
Body weight (and age):	between 369 and 418 g at the start of the experiment (corresponding to an age of about 6 weeks, animals were born on January 5, 1998 shipping slip No. 01195)
Sex:	male
Acclimatization:	6 days. Animals were observed daily to ascertain their fitness for the study
Housing (room H5/A):	2 or 3 animals/cage in an air-conditioned room - temperature: 22 ± 2°C - air changes: about 20/h filtered on HEPA 99.97% - relative humidity: 55 ± 10% - artificial light: 12 h cycle (7 a.m. - 7 p.m.) - cage: wire cages (40.5x38.5x18h) with a stainless steel feeder The waste that dropped through the wire bottom onto a removable paper was periodically disposed of.

Animal identification: by coloring different areas of the ears and paws.
A computerized randomization program was used to allocate the animals to groups.

Cage identification: by cage card giving the experimental number, starting day and group identification in indelible ink.

Diet: the standard GLP diet - certificate coded 8 GP 22, produced by [redacted] feed licensee [redacted], was used.
The declared contents, on the label, on dry matter basis (moisture 12%), were:

crude protein	19 %
crude fat	4 %
crude fiber	14.5 %
crude ash	7.5 %

The diet was supplemented by the Producer with vitamins and trace elements. According to the analytical certificates provided by the Supplier, the contents of the batch of diet used in this study were within ± 5% of the declared values and the presence and the levels of contaminants were within the limits proposed by EPA-TSCA (44FR: 44053-44093, July 26, 1979).
Animal feed, in compliance with [redacted] SOPs, is analyzed twice a year for bacterial contamination.
The diet was available "ad libitum" to the animals.

Water:	filtered water was distributed "ad libitum" to the animals by means of an automatic watering valve system. The drinking water came from the municipal water main. Periodically, drinking water is analyzed to determine microbial count, heavy metals, other contaminants (e.g. solvents, pesticides) and other chemical and physical characteristics. The accepted limits for the quality of drinking water are those defined in EEC Directive 80/778. Contaminants that might interfere with the objectives of the study are not expected to be present either in the diet or in the water. The analytical certificates of the animal feed and water are filed at [redacted] premises.

[redacted]

[redacted]Exp. No.[redacted]

EXPERIMENTAL DESIGN

[redacted]Experiment No.:	[redacted]
Date of preliminary test (tolerability):	February 5-7, 1998
Beginning of the study:	February 16, 1998
End of the study:	March 16, 1998
Experimental groups:	group 1 (treated animals), 10 males numbered from 1 to 10 group 2 (control animals), 5 males numbered from 11 to 15
Administration route:	intradermal injection and topical exposure by occlusive patch
Concentrations used:	0.1% for the intradermal injection 50% for the booster exposure 50% for the challenge application
Volume administered:	0.1 ml/injection site for the intradermal injection 0.3 ml/animal for the booster exposure 0.2 ml/animal for the challenge application
Observation of clinical signs:	daily
Body weight recording:	pre-trial and weekly thereafter

Induction phase: intradermal injection

Day -1: fur was clipped from an area of about 4x6 cm on the shoulder region with an electric clipper before injection.
Care was taken to avoid abrading the skin, which could alter the results of the study.

Day 0: each animal was given three pairs of intradermal injections in the skin area clipped the day before, so that on each side of the midline there was one row of three injections.

The injections were:

Group 1 (test article)
1) 0.1 ml FCA emulsion (1:1 mixture (v/v) FCA/water)
2) 0.1 ml test article
3) 0.1 ml test article in FCA (1:1 mixture (v/v) FCA/water)

In injection 3 the test article was dissolved in water for injection prior to mixing with FCA.
The concentration of the test compound was therefore equal to that used in injection 2.

Group 2 (vehicle)
1) 0.1 ml FCA emulsion (1:1 mixture (v/v) FCA/water)
2) 0.1 ml vehicle
3) 0.1 ml vehicle in FCA (1:1 mixture (v/v) FCA/water)

Twenty-four hours later the injection sites were observed for irritant effects.
The results were recorded.

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Induction phase: booster

Day 5: the area destined to receive the booster (that used for the induction), was clipped and treated with 0.5 ml of 10% sodium lauryl sulfate (Merck, batch L149860, expiry date September 1999) in vaseline oil (Carlo Erba, batch A908393768, expiry date July 2001), in order to create a local irritation and therefore to enhance skin permeability to the compound for the following day.

Day 6: a filter paper (3M Whatman, 2x4 cm) was fully-loaded with the test article or vehicle and applied to the skin areas clipped the day before. The patch was covered by an overlapping impermeable, hypoallergenic, plastic adhesive tape (3M Blenderm). This in turn was firmly secured by adhesive bandage (3M Micropore), wound around the torso of the animal.
The dressing was left in place for 48 hours.

Twenty-four hours after removal of the patches, the patch sites were observed for irritant effencts.
The results were recorded.

Challenge application

Day 19: an area of about 5x5 cm on both the flanks of the animals of the two groups was clipped.

Day 20: an occlusive patch (2x2 cm) loaded with the test article or vehicle were applied for 24 hours to the animals of the two groups.

Left flank: test article
Right flank: vehicle

Day 21: the patches were removed.

Days 22 and 23:	approximately 21 hours after removing the patches the challenge areas were clipped and approximately 3 hours later (48 hours from the start of the challenge patch application) the skin was observed and any reaction recorded (day 22). Twenty-four hours after the above observation a second observation was made and once again recorded (day 23).

[redacted] Exp. No. [redacted]

ASSESSMENT OF SKIN REACTIVITY

Evaluation of skin reactions:

0 absent
1 discrete or patchy erythema
2 moderate and confluent erythema
3 intense erythema and swelling

Animals are considered positive if showing at least discrete or patchy erythema (score=1), while no reaction is seen in the control animals. The sensitization potential of test compound was calculated on the basis of the percentage of animals showing a response of 1 or greater.
The degree of sensitization was evaluated by the maximization procedures which classify it in the following five groups, ranging from weak (grade I) to extreme (grade V) according to the percentage of animals sensitized.

Percentage of animals showing sensitization	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme

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RECORD FILING

The protocol, a reserve sample of the batch of the test article used, the raw data bound in a register numbered [redacted] the final report and all other required document pertinent to the conduct of this study, including records and reports of maintenance, cleaning, calibration and inspection of equipment, will be filed at [redacted] premises for ten years from the issue date of this report and then sent to the Sponsor.

At the end of the ten-year archiving period, the Sponsor can request the extension of the storage of all materials or part of them for a further period. An appropriate agreement will be drawn up accordingly.

PROCEDURAL DETAILS

The study was conducted in accordance with the procedures described in the [redacted] Standard Operating Procedures (SOPs) collection.

Protection of animals used in the experiment is in accordance with Directive 86/609/EEC, enforced by the [redacted] D. L. No. 116 of January 27, 1992.

Physical facilities and equipment for accommodation and care of animals are in accordance with the provisions of EEC Council Directive 86/609.

The Institute is fully authorized by Competent Veterinary Health Authorities.

REFERENCES

(1): Klecak G.
"Identification of contact allergens: predictive tests in animals"
In: Dermatoxicology and Pharmacology, Marzulli F.N. and Maibach H.I. eds., Hemisphere Publishing Co. Washington DC., p. 313 - 316, 1977.

(2): Magnusson B. and Kligman A.M.
"The identification of contact allergens by animal assay. The guinea pig maximization test"
J. Invest. Dermatol., Vol. 52, p. 268-276, 1969.

(3): Magnusson B. and Kligman A.
"Usefulness of guinea pig tests for detection of contact sensitizers".
In: Dermatoxicology and Pharmacology, Marzulli F.N. and Maibach H.I. eds., Hemisphere Publishing Co. Washington DC, p. 551-559, 1977.

RESULTS

MORTALITY, CLINICAL OBSERVATIONS AND BODY WEIGHT

Five test article treated animals died during the second half of the study. Mortalities are given in the following scheme:

Animal No.	Experimental day of death
1	13
3	15
4	14
7	15
9	13

Before death (from day 11 onward) these five animals showed anorexia, they appeared thin, dehydrated and showed hunched posture and piloerection. Severe body weight decrease was also recorded (see Table 1).

Mortalities occurred 7-9 days after the topical application (48 hour duration) in the shoulder region of 0.3 ml of 50% test article concentration (booster).

The transdermal toxicity of the test article was increased by the topical application of sodium lauryl sulphate (0.5 ml/animal of the 10% concentration) at the treatment site on the day preceding the booster (as required by the protocol since the concentration selected for the booster did not result in skin changes in the preliminary test).

The Sodium lauryl sulphate applications clearly increased the permeability of the skin to the test article. In fact at the challenge the surviving animals were again treated with the 50% test article concentration and neither mortality and clinical signs nor local reactions were observed (while severe local reactions occurred in all animals when sodium lauryl sulphate was administered).

At the autopsy in all five animals emaciation, dehydration and empty stomach and empty intestine were found. Skin treatment sites appeared severely inflammed with crusts and desquamation.

The 5 animals surviving animals did not show general clinical signs, but at the skin treatment site severe erythema was observed. Body weight stasis or slight body weight decrease was recorded in 4 out of the 5 animals on days 13 and 19. However an additional body weight recording carried out on day 25 (i.e. after 5 days from the challenge) showed a general trend toward recovery.

EVALUATION OF SKIN SENSITIZATION

Twenty-four hours after the intradermal injections all animals were in good health and, as expected, at each injection site of FCA emulsion (FCA/water), FCA/vehicle and FCA/test article a swollen reddish area was seen. The injection of the test article at the concentration of 0.1% in the vehicle, caused slight irritation. No reaction was seen after injection of the vehicle alone.

Twenty-four hours after the removal of the 48-hour closed patch (booster), signs of severe irritation such as severe redness and thickening of the skin were observed in animals treated with the test article, while in controls slight skin thickening was observed as consequence of the sodium lauryl sulphate application.

Table 2 shows the results obtained at the challenge exposure in surviving animals with the test article [redacted] applied by an occlusive patch at the concentration of 50%.

No animals showed positive reactions at the challenge.

No skin reactivity was observed in the negative control group (see Table 3).

CONCLUSIONS

Contact sensitivity is a T-lymphocyte-mediated delayed hypersensitivity reaction.

The immunological events in skin sensitization can be separated into two main phases: development of sensitization and elicitation of clinical effects (e.g. erythema and edema) following subsequent exposure to the same compound.

The sensitizing potential of the test article [redacted] was assessed in guinea-pigs using the Magnusson test as described by Klecak (1), Magnusson B. and Kligman A. M. (2, 3).

All test article treated animals showed severe local irritation and half of them died within 7-9 days of the booster (topical application of 50% test article concentration). Local reactions and mortality were considered to be caused by increased transdermal absorption of the test article after sodium lauryl sulphate (SLS) application on the skin treatment sites on the day preceding the booster.

The death was preceded by anorexia, body weight loss, dehydration, hunched posture and piloerection. At autopsy empty stomach and empty intestine, dehydration, and emaciation were found. On skin treatment sites severe inflammation, with crusts and desquamation, was seen.

Surviving animals only showed body weight stasis or slight body weight decrease and local severe erythema and skin thickening. Body weight showed an evident trend towards recoveryat the end of the study.

No surviving animals treated with the test article showed either general and local clinical signs or a positive reaction at the challenge (topical application of 50% test article concentration without SLS application).

On the basis of this result, under the experimental conditions applied, [redacted] did not appear to possess sensitizing capacity; however, it should be considered that assesment was done on a limited (surviving) number of animals.

Dr. [redacted] 21/4/88
[redacted] tudy Director

Dr. [redacted] 21/4/88
[redacted] Senior Scientist for General Toxicology

[redacted] Exp. No [redacted]

TABLES

[redacted] Exp. No. [redacted]

Test article: [redacted]
Title : Skin sensitization test in guinea-pigs
[redacted] exp. : [redacted]

TABLE 1. - Body weight
(expressed in grams)

Group No.	Guinea-pig No.	Days				
		-1	5	13	19	25
1	1	389	419			
	2	369	433	411	415	493
	3	389	446	449		
	4	391	454	311		
	5	404	467	491	496	549
	6	381	446	463	514	556
	7	379	416	380		
	8	412	450	481	489	523
	9	418	473			
	10	395	450	436	440	509
2	11	389	414	461	497	552
	12	389	443	508	512	580
	13	391	433	507	567	612
	14	399	438	473	514	545
	15	387	414	463	525	567

Animal No. 1 found dead on day 13
Animal No. 3 found dead on day 15
Animal No. 4 found dead on day 14
Animal No. 7 found dead on day 15
Animal No. 9 found dead on day 13

[redacted] Exp. No. [redacted]

Test article: [redacted]
Title : Skin sensitization test in guinea-pigs
[redacted] exp. : [redacted]
Vehicle : deionized water

TABLE 2. - Challenge in surviving treated animals

	Challenge			
	Day 22		Day 23	
Group 1 Guinea pig no.	Test article	Vehicle	Test article	Vehicle
2	0	0	0	0
5	0	0	0	0
6	0	0	0	0
8	0	0	0	0
10	0	0	0	0

Skin reaction was assessed according to the scores described in the test (see section assessment of skin reactivity)

No. of positive animals at the challenge: 0

Result: **NEGATIVE**

[redacted] Exp. No. [redacted]

Test article: [redacted]
Title : Skin sensitization test in guinea-pigs
[redacted] exp. : [redacted]
Vehicle : deionized water

TABLE 3. - Challenge in control animals

	Challenge			
	Day 22		Day 23	
Group 2 Guinea pig no.	Test article	Vehicle	Test article	Vehicle
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0

Skin reaction was assessed according to the scores described in the test (see section assessment of skin reactivity)

No. of positive animals at the challenge: 0

Result: **NEGATIVE**

[redacted] Exp. No. [redacted]

ATTACHMENT

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[redacted]

Attachment No. 1 (p.1)

SENSITIVITY CHECK OF THE DUNKIN HARTLEY GUINEA-PIG IN THE SKIN SENSITIZATION STUDY

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[redacted]

Attachment No. 1 (p.2)

TITLE

Sensitivity check of the Dunkin-Hartley guinea-pig in the skin sensitization study.

PURPOSE

To assure guinea-pigs' continuing responsiveness and that the technical aspects of the procedure are being correctly followed.

This sensitivity check is required by the pertinent OECD and EEC Guidelines.

TEST METHOD

Magnusson's maximization test was followed.

The test method is in accordance with method B.6, Annex V to Directive 67/548 (EEC Directive 96/54, EEC Official Journal, No. L 248, September 30, 1996) and with Organization for Economic Cooperation and Development (OECD) Guidelines (section 4, subpart 406, Paris 1981 and subsequent updatings).

PROCEDURAL DETAILS

The test is conducted in compliance with the OECD-GLP in the testing of chemicals, [C(81) 30 (final)], regulations also enforced by the [redacted] Health Authority [D.M. dated June 26, 1986 as published in G.U. No. 198, dated August 27, 1986 and D.L. January 27, 1992, No. 120, as published in G.U. (Supplement) No. 40, February 18, 1992].

EXPERIMENTAL DATE

The study was conducted in December 1997/January 1998.

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POSITIVE CONTROL

Identification:	2,4- dinitrochlorobenzene (DNCB) chosen on the basis of the EEC Guideline indication
Batch No.:	VV 218627
Expiry date:	May 2000
Producer:	Merck (Germany)
Vehicles:	vaselin oil

CONCENTRATIONS USED

Magnusson test:	0.02% for the intradermal injection 0.4% for the booster exposure 0.2% for the challenge application

FORMULATE PREPARATION

Intradermal injection

DNCB was dissolved in vaselin oil to obtain the 0.02% concentration.

For the injection in Freund's Complete Adjuvant (FCA) DNCB was dissolved in FCA at the concentration of 0.04% and then an equal volume of water for injection was added to obtain the final concentration of 0.02%.

Topical application

Test article concentrations 0.4% and 0.2% in vaseline oil were prepared for the booster and the challenge, respectively.

[redacted]

Attachment No. 1 (p. 4)

TEST DESCRIPTION

Magnusson test:	2 administrations, one per week, the first one by intradermal injection, the second one by 48h patch application (induction phase), and one exposure, 24h patch, 14 days after the last induction phase (challenge application).

TEST SYSTEM

Species and strain:	Dunkin Hartley albino guinea-pigs
Number and sex:	5 males treated with the positive control
Supplier:	[redacted]
Housing:	2 or 3 animals/cage in an air-conditioned room - temperature: 22 ± 2°C - air changes: about 20/h filtered on HEPA 99.97% - relative humidity: 55 ± 10% - artificial light: 12 h cycle (7 a.m. - 7 p.m.) - cage: wire cages (40.5x38.5x18h) with a stainless steel feeder
Animal identification:	by coloring different areas of the ears and paws.
Diet:	standard GLP diet - certificate coded 8 GP 22 (produced by [redacted] feed licensee [redacted]
Water:	"ad libitum".

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Attachment No. 1 (p. 5)

TABLE 1. - Skin sensitization test in guinea-pigs (Magnusson test)

	Challenge			
	Day 22		Day 23	
Guinea pig no.	Positive control	Vehicle	Positive control	Vehicle
1	2	0	1	0
2	2	0	1	0
3	3	0	2	0
4	3	0	2	0
5	3	0	2	0

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